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Differential Transcription of Pea Chloroplast Genes during Light-Induced Leaf Development[1]

Continuous Far-Red Light Activates Chloroplast Transcription

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Plastid gene expression was analyzed in pea (*Pisum sativum* L.) plants grown in darkness, continuous far-red light, and white light. Responses induced by continuous far-red light were most likely mediated by PHYA. Plastid transcription activity was low in dark-grown plants. In contrast, plastids of plants grown in white or far-red light showed a 10-fold increase in transcription activity between 4 and 6 d postimbibition (dpi) and a decrease between 6 and 9 dpi. Plastid RNAs accumulated in illuminated plants from 5 to 7 dpi. In far-red-light-illuminated plants, plastid mRNA levels remained elevated until 14 dpi. In white-light-grown plants, most plastid RNAs decreased in abundance after 7 dpi to very low levels by 14 dpi. This indicates that white light induces a general decrease in plastid RNA stability compared to far-red-light-illuminated seedlings. *PsbA* mRNA accumulated in older, dark-grown, far-red, and white-light-illuminated seedlings, consistent with this RNA having high stability. Transcription of genes encoding the plastid's transcription and translation apparatus increased relative to *rbc*L and other genes encoding proteins of the photosynthetic apparatus from 4 to 5 dpi and then declined 10-fold from 5 to 9 dpi. These data document dynamic modulation of plastid gene transcription and mRNA stability during light-induced chloroplast development in pea.

The chloroplast genome encodes approximately 120 genes (reviewed by Igloi and Kössel, 1992; Gruissem and Tonkyn, 1993; Mullet, 1993). Some of these genes encode RNAs (rRNA, tRNA) and proteins that contribute to the plastid's transcription and translation apparatus. For example, *rpo*A, B, C1, and C2 encode subunits of an RNA polymerase, and *rps*16 and *rpl*16 encode subunits of the plastid ribosome. Another large category of plastid genes encode subunits of the photosynthetic apparatus. These include *rbc*L (Rubisco large subunit); *psa*A, *psa*B, *psa*C (PSI complex); *psb*A-I, K, and L (PSII complex); *pet*A, B, and D (Cyt complex), and atpA, B, E, F, H, and I (ATP synthase). The plastid genes are often organized in complex operons that contain multiple promoters and RNA-processing sites. For example, the barley *psbD-psbC* operon includes *trn*G, *orf*62, *psb*K, *psb*I, and *trn*S (Sexton et al., 1990a, 1990b). Genes in this operon are transcribed from at least four different promoters, including one that is light inducible (Sexton et al., 1990a). RNA processing and transcription termination create four different 3′ termini resulting in more than 15 different transcripts. Some operons contain genes encoding the transcription apparatus (*rpo*B-*rpo*C1-*rpo*C2) and ribosomes (rRNA operon, *rps*16, *rpl*16-*rpo*A operon) but not genes for photosynthesis. Others contain genes for proteins of the photosynthetic apparatus (*rbcL-atp*B, *psb*B-*psb*H-*pet*B-*pet*D). This organization, in principle, offers the opportunity to differentially regulate transcription of these genes during chloroplast development. In barley, differential transcription of genes encoding the transcription/translation apparatus versus genes encoding the photosynthetic apparatus occurs early in chloroplast development (Rapp et al., 1992). In addition, expression of most plastid genes is regulated at the level of RNA processing and RNA stability (reviewed by Gruissem and Tonkyn, 1993).

Transcription of the plastid genes is mediated by at least two RNA polymerases (Greenberg et al., 1984; Rajasekhar et al., 1991; Hess et al., 1993; Lerbs-Mache, 1993) and is modulated by σ-like factors (Bülow and Link, 1988; Tiller et al., 1991; Troxler et al., 1994). One of the plastid RNA polymerases is encoded by the plastid genome (*rpo*A, B, C1, C2). This RNA polymerase is thought to recognize plastid promoters containing prokaryotic −10 and −35 sequence motifs (TATA and TTGACA, respectively). The second plastid-localized RNA polymerase is less well characterized but has been reported to recognize promoters that lack the −35 motif (Lerbs-Mache, 1993). This second RNA polymerase contains a 110-kD subunit, and subunit(s) of this polymerase are encoded by nuclear gene(s) (Lerbs-Mache, 1993).

In barley, activation of plastid transcription is light independent and occurs early in chloroplast development when cells stop dividing and enter the zone of cell elongation (Baumgartner et al., 1989). Activation of plastid transcription in barley leads to the accumulation of plastid RNAs and most of the proteins found in mature chloroplasts (reviewed Mullet, 1993). In pea (*Pisum sativum* L.), low levels of plastid transcripts accumulate in leaves of

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Abbreviations: dpi, days postimbibition; PHY, phytochrome.

dark-grown plants, and light induces accumulation of plastid RNAs (Thompson et al., 1983; Bennett et al., 1984; Sasaki et al., 1984; Woodbury et al., 1988). Plastid transcription can be activated by red or far-red light, and activation by red light is not reversed by far-red-light illumination (Bottomley, 1970; Bennett et al., 1984). Therefore, activation of plastid transcription is probably controlled in part by a very-low-fluence response mediated by PHY.

In this study we examined the relationship between pea leaf growth in darkness and far-red and white light and steps in chloroplast development, including activation of plastid RNA synthesis and accumulation of RNA and Chl. This analysis shows that RNA synthesis in plastids is activated by white or far-red light early in leaf development and that far-red light stimulates transcription more than white light. In addition, evidence for light-induced differential transcription of certain plastid genes was obtained.

MATERIALS AND METHODS

Plant Growth

Pea (*Pisum sativum* L. var Little Marvel) seeds were allowed to imbibe in distilled water for 8 h with continuous shaking and planted in vermiculite saturated with full-strength Hoagland solution at a depth of 2 cm. Seedlings were watered with full-strength Hoagland solution and grown in controlled environmental chambers at 23°C. Seedlings were transferred to continuous white-light (350 μE m^{-2} s^{-1}) or continuous far-red-light (30 μE m^{-2} s^{-1}) chambers 2 d after planting or left in darkness throughout the growth period.

Light Sources

Far-red light was obtained by filtering the output of a 60-W incandescent lamp through a CBS FR 750 filter (Carolina Biological Supply, Burlington, NC). The far-red-light fluence rate was modified by use of a rheostat and was measured with a Li-Cor 1800 spectroradiometer (Lincoln, NE). Continuous white light was obtained from the combined output of 60-W incandescent and 150-W fluorescent bulbs. The total white-light fluence rate was determined with a Li-Cor LI-185B quantum radiometer/photometer. The far-red light (700–800 nm) using this white-light source was 56.94 $\pm$ 0.5 μE m^{-2} s^{-1}.

Plastid Isolation and Chl Determinations

Chloroplasts from pea primary leaves were isolated according to the method of Baumgartner et al. (1989). For plants grown in white light, Percoll gradients consisted of an upper layer containing 40% (v/v) Percoll and a lower layer with 85% (v/v) Percoll. Plastids isolated from plants grown under continuous far-red light were purified on 35 and 75% Percoll gradients. Plastids isolated from dark-grown plants were purified on 30 and 65% Percoll gradients. Chl was extracted from a known number of chloroplasts with 80% (v/v) acetone as described by Arnon (1949).

Plastid Transcription Assays and RNA Blots

RNA was extracted from isolated plastids, separated on formaldehyde-agarose gels, blotted onto GeneScreen Plus membranes, and probed with in vitro antisense probes as described by Rapp et al. (1992). The probes for RNA blots and transcription assays were identical with those used by Rapp et al. (1992). Plastid run-on transcription assays (Mullet and Klein, 1987) were carried out as modified by Rapp et al. (1992). Radiolabeled transcripts generated in 10-min lysed run-on transcription assays were extracted as described by Mullet and Klein (1987). The transcripts were hybridized to dot blots containing saturating levels (1 pmol) of in vitro synthesized antisense RNA transcripts of each analyzed gene.

RESULTS

Plastid Transcription Activity and Leaf Development

Transcription activity in pea plastids was quantified using run-on transcription assays in the presence of heparin (Fig. 1A) (Rapp et al., 1992). Transcription activity in lysed plastids was insensitive to chloramphenicol and rifampicin but sensitive to tagetitoxin and DNase treatment, similar to barley plastids (Table I) (Mullet and Klein, 1987). Heparin was included to prevent reinitiation by soluble RNA polymerase and to prevent RNA degradation during the pulse-labeling period (Klein and Mullet, 1990). Total transcription activity in plastids of dark-grown plants remained low throughout the developmental period examined. In white-light-illuminated plants, plastid transcription was low 4 dpi, but increased by 5 dpi, and reached peak activity 6 dpi. Plastid transcription activity gradually declined from 6 to 9 dpi and remained low thereafter. In far-red-light-illuminated seedlings, plastid transcription was lower at 5 dpi than in white-light-grown plants. However, by 6 dpi, plastid transcription activity was nearly double that observed in chloroplasts of white-light-illuminated seedlings. When maximal transcription activity per plastid was reached at 6 dpi, leaves were 6.4 mm long or approximately 25% of full size (DuBell and Mullet, 1995). Plastid transcription activity in far-red-light-illuminated seedlings declined from 6 to 9 dpi as observed in white-light-grown plants.

Accumulation of Chl

The accumulation of Chl in plastids as a function of leaf development in white-light-grown pea plants was determined to provide a measure of the buildup of the photosynthetic apparatus. The results in Figure 1B show that little Chl accumulates in plastids prior to 5 dpi. Between 5 and 7 dpi, Chl content per chloroplast reached 50% of the maximum observed. Accumulation of Chl occurred more slowly per chloroplast from 7 to 11 dpi and then decreased. The decline in Chl starting at 12 dpi may correspond to the initiation of leaf senescence.

Analysis of Plastid RNAs

Transcripts corresponding to 14 different plastid genes were analyzed using the RNA blots shown in Figure 2.

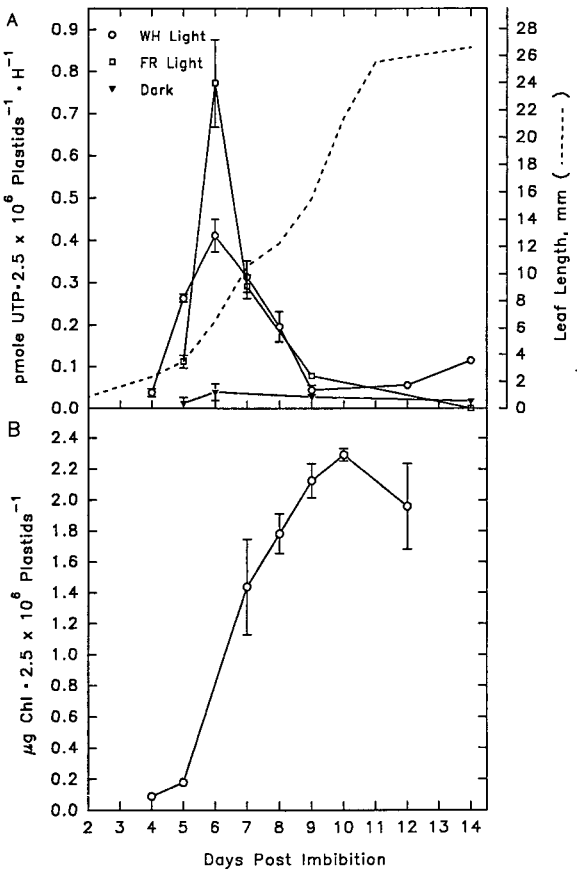

Figure 1. Changes in transcription activity and accumulation of Chl in developing chloroplasts. A, Transcription activity of plastids isolated from pea plants grown in darkness or continuous white or far-red light. Transcription assays were performed as described in "Materials and Methods." Each data point represents the mean ± SE of two experiments. B, Chl content of chloroplasts isolated from pea plants grown in continuous white light. Each data point represents the mean ± SE of two experiments.

Plastids were isolated from plants grown for 5, 7, or 14 d in darkness or far-red or white light. RNA was separated on gels, transferred to membranes, and probed with gene-specific RNA probes (Rapp et al., 1992). The RNA blots in Figure 2A correspond to genes encoding 16S rRNA, tRNA-fMet, tRNA-Gly, and tRNA-Lys. 16S rRNA could be detected in plastids of dark-grown seedlings at 5 dpi, and the level of 16S rRNA increased slowly up to 14 dpi. The level of 16S rRNA in far-red-light-illuminated seedlings at 5 dpi was higher than in dark-grown seedlings but similar to seedlings grown in white light. There was an increase in rRNA abundance by 7 dpi in far-red light and a small decrease in abundance by 14 dpi. In contrast, 16S rRNA levels decreased between 5 and 7 dpi in white-light-grown seedlings, and levels were much lower at 14 dpi. Changes in the abundance of RNA hybridizing to *trn*fM/G and *trn*K showed a similar pattern.

The RNA blots in Figure 2B correspond to genes that encode subunits of the plastid-encoded RNA polymerase (*rpo*A, *rpo*B) and plastid ribosomes (*rpl*16, *rps*16). In seedlings grown in darkness or far-red light, *rpo*A and *rps*16 mRNAs were low in plastids of 5-d-old seedlings, increased at 7 dpi, and remained high through 14 dpi. In contrast, *rpo*B transcript abundance increased between 5 and 7 dpi in dark-grown and far-red-light-illuminated seedlings and declined again by 14 dpi in dark-grown plants. *Rpl*16 transcript abundance was already high at 5 dpi in dark-grown seedlings, and the levels of this RNA declined by 14 dpi. In white-light-grown seedlings, the highest levels of these RNAs occurred at 5 dpi with very low levels detected by 14 dpi.

Figure 2, C and D, shows RNA blots corresponding to *ndh*A and six genes encoding proteins of the photosynthetic apparatus. In general, the transcript levels for all of these genes were higher in far-red- and white-light-illuminated seedlings compared to dark-grown seedlings. In far-red-light-grown plants, with the exception of *psb*A, RNA levels encoding proteins of the photosynthetic apparatus genes peaked at 7 dpi and gradually declined 2- or 3-fold by 14 dpi. In white-light-grown plants, these same plastid mRNAs showed highest abundance at 7 dpi and then declined dramatically by 14 dpi. In contrast, *psb*A mRNA abundance increased steadily from 5 to 14 dpi in all growth conditions.

Transcription of Individual Plastid Genes

Plants grown in white light showed maximal levels of plastid rRNA, tRNA, and mRNA for genes encoding the transcription and translation apparatus at 5 dpi. In contrast, mRNAs corresponding to genes encoding subunits of the photosynthetic apparatus showed maximal abundance at 7 dpi or later (i.e. *psb*A). In barley, transcription of these two classes of genes also showed different kinetics of change during chloroplast development (Baumgartner et al., 1993). To examine this possibility in pea, transcription run-on assays were performed, and transcription from individual genes was assayed using gene-specific antisense probes. The results of these assays are shown in Table II. To detect more easily differential transcription, transcription activity is expressed relative to *rbc*L at each developmental

Table I. *Characterization of transcription in lysed plastids*

Plastids were isolated from 6-d-old light-grown pea plants. Transcription in lysed plastids was performed as described in "Materials and Methods" with the below modifications. Exogenous DNA was plasmid pPPBX-10218 containing the plastid *psb*A promoter (Boyer and Mullet, 1986).

Treatment	[^{32}P]UTP Incorporation
	%
Control	100
Glycerol (4%)	98.6 ± 5.6
Chloramphenicol (100 µg/mL)	103.8 ± 0.5
Rifampicin (20 µg/mL)	101.7 ± 0.9
Rifampicin (100 µg/mL)	109.2 ± 0.4
Tagetitoxin (80 µM)	19.8 ± 8.3
DNase I[a] (1 unit)	1.4 ± 0.4
Exogenous DNA (1 µg)	100.5 ± 6.0
Spermidine (100 µM)	99.7 ± 7.1

[a] Total plastid membranes were incubated with DNase I for 15 min at 4°C prior to addition to the transcription assay.

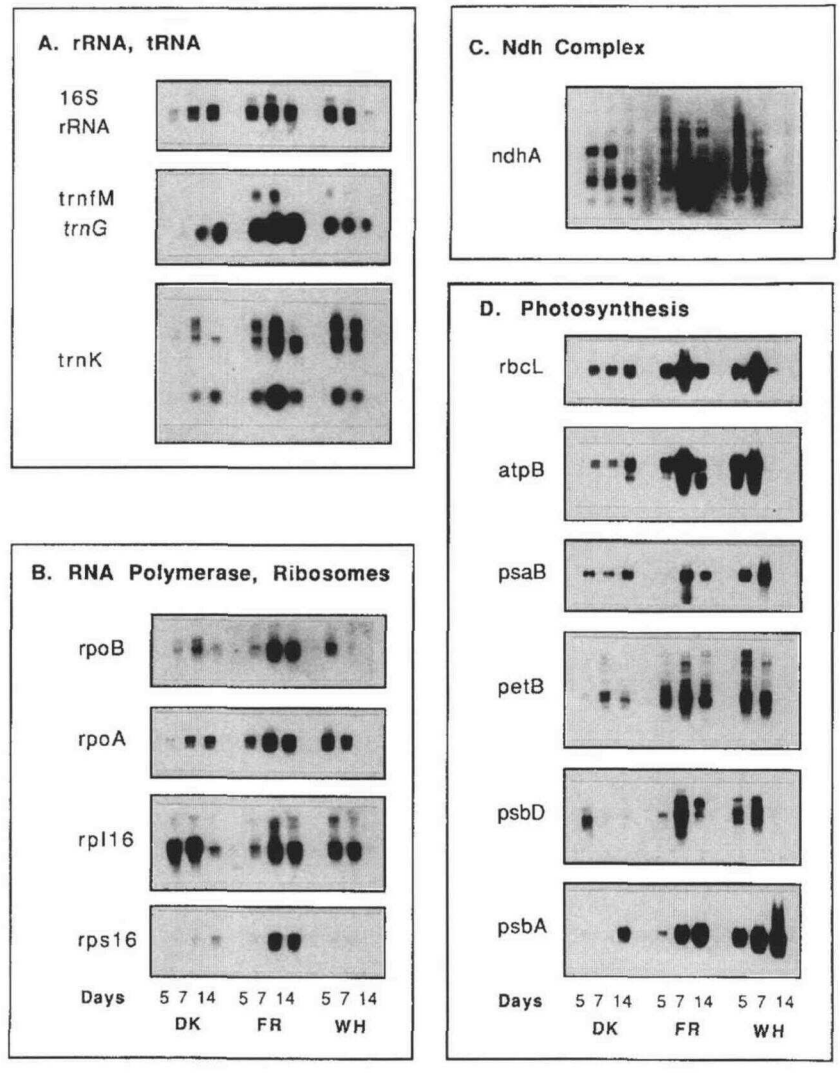

Figure 2. RNA accumulation in developing plastids. Plastids were isolated from primary leaves of peas grown 5, 7, or 14 d in darkness (DK), continuous far-red light (FR), or white light (WH). RNA from 1×10^7 plastids was loaded in each lane and probed with antisense RNA from the gene shown at the left. A, Genes encoding rRNA and tRNAs (16S rRNA, *trnf*M/*trn*G, *trn*K). B, Genes encoding subunits of the plastid-encoded RNA polymerase and ribosomal proteins (*rpo*B, *rpo*A, *rpl*16, *rps*16). C, A gene encoding a protein of the NADH oxidoreductase complex (*ndh*A). D, Genes encoding proteins of the photosynthetic apparatus (*rbc*L, *atp*B, *psa*B, *pet*B, *psb*D, *psb*A).

stage examined. In addition, changes in the ratio of a gene's transcription relative to *rbc*L from 4 to 5 dpi and from 5 to 9 dpi were calculated and are listed on the right of Table II.

In general, the ratio of transcription of genes encoding proteins of the photosynthetic apparatus and *trn*K was constant from 4 to 5 dpi and varied no more than 2.7-fold between 5 and 9 dpi. Transcription of *psb*D increased relative to *rbc*L from 5 to 9 dpi. The increase in *psb*D transcription is consistent with the presence of a light-inducible promoter in the *psb*D operon (Sexton et al., 1990a). In contrast, transcription of 16S rRNA, *trn*fM/G, *rpo*A, *rpo*B, *rps*16, and *rpl*16 increased 1.7- to 3.3-fold relative to *rbc*L from 4 to 5 dpi and decreased 4.5- to more than 10-fold from 5 to 14 dpi. Transcription of *ndh*A showed an intermediate response but decreased 6.5-fold relative to *rbc*L from 5 to 9 dpi.

Transcription of plastid genes in plants grown in far-red light at 5 and 7 dpi was also analyzed (Table III). The relative ratio of transcription of genes in far-red- and white-light-grown plants at 5 dpi was similar. Between 5 and 7 dpi in far-red-light-grown plants, transcription of *rpl*16, *rpo*B, *ndh*A, and 16S rRNA decreased approximately 2-fold relative to *rbc*L. Similar decreases in relative transcription of these genes were observed in white-light-grown plants. However, transcription of *rps*16 and *rpo*A did not decrease relative to *rbc*L between 5 and 7 dpi in far-red-light-grown plants, whereas in white-light-illuminated plants a decrease in relative transcription was observed (Table III).

DISCUSSION

Regulation of Total Plastid Transcription Activity during Pea Leaf Development

The overall transcription activity in pea plastids of dark-grown plants remained low from 4 to 14 dpi. Leaf growth in darkness was also slow relative to illuminated plants. Based on analysis of leaf and cell size, cell number, DNA content per leaf, and plastid number per cell, it can be estimated that the number of plastids per leaf of dark-grown plants increased 4-fold from 4 to 12 dpi, whereas in

Table II. *Ratio of plastid gene transcription relative to rbcL transcription during pea chloroplast development in continuous white light*

Ratio of transcription of the plastid genes listed on the left relative to *rbc*L from leaves grown for 4, 5, 7, and 9 d in continuous white light (WL). The fold change in the ratio of the transcription of each gene was calculated using the values from 4, 5, and 9 d in white light. NC, No change in ratio; ND, not determined.

Gene	Developmental Stage				Fold Change in Ratio	
	4 d WL	5 d WL	7 d WL	9 d WL	5 d WL / 4 d WL	9 d WL / 5 d WL
16S rRNA	11.64 ± 0.26	21.82 ± 0.37	12.20 ± 0.55	2.3 ± 0.95	↑ 1.9	↓ 9.2
*trn*fM/G	0.59 ± 0.006	0.73 ± 0.63	0.63 ± 0.006	0.16 ± 0.002	NC	↓ 4.5
*trn*K	0.88 ± 0.03	0.88 ± 0.02	0.75 ± 0.05	0.52 ± 0.02	NC	↓ 1.7
*rpo*B	0.17 ± 0.01	0.29 ± 0.01	0.17 ± 0.006	0.03 ± 0.009	↑ 1.7	↓ 9.6
*rpo*A	0.06 ± 0.008	0.20 ± 0.04	0.03 ± 0.003	0.002 ± 0.01	↑ 3.3	↓ 100
*rpl*16	0.05 ± 0.000	0.16 ± 0.02	0.09 ± 0.02	ND	↑ 3.2	ND
*rps*16	0.09 ± 0.008	0.22 ± 0.01	0.12 ± 0.002	0.03 ± 0.006	↑ 2.4	↓ 7.3
*ndh*A	0.09 ± 0.003	0.13 ± 0.02	0.13 ± 0.01	0.02 ± 0.002	NC	↓ 6.5
*rbc*L	1.00 ± 0.04	1.00 ± 0.15	1.00 ± 0.006	1.00 ± 0.02	NC	NC
*atp*B	ND	0.31 ± 0.005	0.24 ± 0.001	0.13 ± 0.008	ND	↓ 2.3
*psa*B	1.63 ± 0.009	2.07 ± 0.05	1.69 ± 0.09	0.75 ± 0.02	NC	↓ 2.7
*pet*B	0.26 ± 0.005	0.32 ± 0.002	0.25 ± 0.002	0.12 ± 0.01	NC	↓ 2.5
*psb*D	0.45 ± 0.004	0.41 ± 0.003	0.54 ± 0.02	0.71 ± 0.03	NC	↑ 1.7
*psb*A	6.12 ± 0.30	6.83 ± 0.15	5.59 ± 0.30	4.97 ± 0.01	NC	↓ 1.4

white light a 50-fold increase occurred and in far-red light a 20-fold increase occurred (DuBell and Mullet, 1995). Therefore, the increase in transcription activity in plastids of illuminated plants relative to dark-grown plants is due, in part, to the need to synthesize the photosynthetic apparatus in the population of plastids present in leaves of 4-d-old plants and to build up the total number of chloroplasts per leaf. The initial activation of plastid transcription in pea plastids occurred between 4 and 5 dpi in plants grown in white light, and maximal transcription rates were measured in chloroplasts of 6-d-old plants. In far-red-light-illuminated seedlings, the increase in transcription was delayed slightly, but total activity per plastid peaked at 6 dpi and was higher than in white-light-illuminated plants. Because plastid number per cell at 6 dpi was the same in plants grown in white and far-red light, plastid transcription per cell and per leaf was higher in this condition.

Table III. *Ratio of plastid gene transcription relative to rbcL transcription during pea chloroplast development in continuous far-red light*

Ratio of transcription of the plastid genes relative to *rbc*L in pea leaves grown for 5 and 7 d in continuous far-red light (FR).

Gene	Relative Transcription Rates	
	5 d FR	7 d FR
16S rRNA	20.06 ± 0.83	12.58 ± 0.48
*trn*fM/G	0.84 ± 0.02	0.69 ± 0.06
*trn*K	1.13 ± 0.03	0.84 ± 0.16
*rpo*B	0.29 ± 0.03	0.13 ± 0.01
*rpo*A	0.10 ± 0.01	0.10 ± 0.02
*rpl*16	0.16 ± 0.03	0.08 ± 0.03
*rps*16	0.14 ± 0.02	0.13 ± 0.03
*ndh*A	0.20 ± 0.02	0.10 ± 0.02
*rbc*L	1.00 ± 0.009	1.00 ± 0.07
*atp*B	0.34 ± 0.05	0.37 ± 0.02
*psa*B	2.42 ± 0.28	2.29 ± 0.10
*pet*B	0.30 ± 0.01	0.18 ± 0.02
*psb*D	0.61 ± 0.23	0.63 ± 0.02
*psb*A	7.16 ± 0.29	5.97 ± 0.23

The 10-fold increase in transcription activity per plastid that occurred between 4 and 6 dpi in white-light-illuminated plants was followed by a 10-fold decrease in activity per plastid by 10 dpi. Because plastid number per leaf increased 6-fold during this same period, much of the decrease can be accounted for by dilution of the transcription apparatus into newly synthesized plastids. This observation implies that pea leaves synthesize a large amount of the transcription apparatus early in leaf development in anticipation of the production of additional leaf cells and chloroplasts during subsequent leaf development. In fact, only about 5% of the final leaf Chl accumulated by 6 dpi. A similar activation of plastid transcription is observed early in barley chloroplast development (Baumgartner et al., 1989). In barley, transcription activity per plastid begins to increase as cell division rates decline and peaks in the zone of cell elongation. Transcription activity per plastid rapidly declines in illuminated barley plants without a change in cell number and only a 2-fold change in plastid number per cell (Baumgartner et al., 1989). In pea, dilution of the transcription apparatus into new cells and plastids appears to play a larger role in the observed reduction of transcription activity per plastid during chloroplast development.

Regulation of RNA Synthesis by Continuous Far-Red Light (PHYA)

Continuous far-red light previously has been shown to stimulate PHYA-mediated high-irradiance responses (McCormac et al., 1992, 1993; Whitelam et al., 1992; Parks and Quail, 1993). Plastid transcription and RNA accumulation and plastid DNA synthesis and DNA accumulation (DuBell and Mullet, 1995) were enhanced in pea plants exposed to continuous far-red light. The activation of these processes occurs between 5 and 6 dpi. The time course of

activation of plastid DNA synthesis and transcription is similar in far-red and white light, although greater activity is observed in plants exposed to far-red light. Continuous far-red light has also been shown to strongly induce ascorbate oxidase (Drumm et al., 1972) and Phe ammonia-lyase (Schopfer and Mohr, 1972) activities in mustard cotyledons. In white-light-grown plants, the influence of PHYA may normally be balanced through the action of another photoreceptor.

Differential Expression of Plastid Genes during Leaf Development

The plastid genome in pea is 120 kb in size and its coding capacity is similar to other plant plastid genomes (Woodbury et al., 1988, 1989). The pea genome lacks an inverted repeat found in many other plastid genomes and has also undergone several rearrangements relative to other plastid genomes (reviewed by Palmer, 1985). However, with only a few exceptions such as the *psb*D-*psb*C operon (Christopher et al., 1992), operons in the plastid genome are similar to operons in other plastid genomes, including barley. Previous studies have characterized several pea chloroplast operons and many of the sites of transcription initiation in the pea genome (Woodbury et al., 1992). It was noted previously that the average size of plastid transcripts decreased during chloroplast development probably as a consequence of a decrease in transcription relative to RNA processing (Woodbury et al., 1988).

The level of RNA in plastids of dark-grown plants was lower than in plastids of plants illuminated with far-red or white light. This is not surprising because these plastids showed reduced overall plastid transcription activity. The RNA levels of many of the genes assayed increased gradually in dark-grown plants from 5 to 14 dpi. The RNA levels of a few genes such as *pet*B, *psb*D, *rpl*16, and *rpo*B decreased from 7 to 14 dpi. For example, *rpl*16 transcripts were unusually high in plastids of dark-grown plants at 5 and 7 dpi and decreased greatly by 14 dpi. *trn*E transcripts were also high in plastids of dark-grown plants at 5 and 7 dpi but decreased only slightly by 14 dpi (A.N. DuBell, S. Berry-Lowe, and J.E. Mullet, unpublished data). The elevated level of *trn*E transcripts may be necessary for use in Chl biosynthesis (Berry-Lowe, 1987). *Psb*A RNAs, in contrast, accumulated between 7 and 14 dpi in dark-grown seedlings.

Growth of seedlings in continuous far-red light enhanced plastid transcription activity between 5 and 7 dpi and increased the abundance of plastid RNAs in these plants relative to dark-grown and white-light-illuminated seedlings. At 5 dpi, many plastid RNAs in far-red-light-grown plants were less abundant than in white-light-grown seedlings, but by 7 dpi, RNAs in far-red-light-illuminated plants were higher than in white-light-illuminated plants. This is consistent with an earlier increase in plastid transcription in white-light-grown plants but higher transcription in far-red-light-grown plants at 6 dpi. Plastid transcription activity decreased after 6 dpi in white-light- and far-red-light-illuminated seedlings. The decline in transcription cannot be due to photosynthesis because it is observed in far-red-light-grown seedlings. Turgeon (1984) has shown that leaves switch from net importers to exporters when leaves are 30% of full size. This is approximately the leaf stage at which plastid transcription decreases. It is possible that changes in leaf metabolism or hormone status during the import/export switch also modulate plastid transcription.

Plastid RNA levels were much greater in plants grown in continuous far-red light compared to white-light-grown plants at 14 dpi. This difference in plastid RNA level cannot be explained by transcription because transcription activity in both plastid populations was similar and decreased in parallel from 7 to 14 dpi. Plastid number per cell doubled in white light but not in far-red light during this time; however, this can explain only a small part of the difference in RNA levels. We conclude that growth of plants in white light from 7 to 14 dpi results in a decrease in the stability of most plastid RNAs. This change is particularly dramatic in the case of 16S rRNA, which is normally a very stable RNA (Kim et al., 1993). The decline of pea ribosomes during light-induced chloroplast development in pea was previously reported (Fish and Jagendorf, 1982). Most plastid proteins are very stable; therefore, there is little reason to maintain the full capacity to synthesize plastid proteins once leaf and chloroplast development is complete. The decrease in RNA stability occurs during the final phase of chloroplast development when leaves are acquiring photosynthetic competence. The decrease does not occur in plants grown in far-red light, even though this treatment induced considerable leaf development. Therefore, it is possible that white light acting through photosynthesis or some other photoreceptor activates the turnover of plastid RNAs and ribosomes at this stage of leaf development.

The decrease in RNA stability observed in white light may be greater for some RNAs than others. For example, *rps*16 and *rpo*B mRNAs are relatively abundant in far-red-light-illuminated plants at 7 dpi, whereas these RNAs are nearly undetectable in white-light-grown plants of a similar age. Overall transcription per plastid in these two plants is similar, as is the ratio of *rps*16 or *rpo*B transcription to *rbc*L at 7 dpi. A rapid decrease in *rpo*B mRNA level is also observed when dark-grown plants are exposed to white light, even though *atp*B mRNA levels are increasing in this condition. These results indicate that *rps*16 and *rpo*B RNA abundance is modulated, in part, through changes in RNA stability and that this contributes to relatively rapid changes in RNA levels for these RNAs. It is interesting to note that in barley, *rpo*B mRNA is 8- to 30-fold more abundant than *rpo*A mRNA and *rps*16 mRNA levels are 3- to 8-fold greater than *rpl*16 mRNA levels. Although absolute levels of plastid RNAs were not determined in this study, a similar difference in mRNA abundance is predicted based on x-ray film exposure time. This is significant because the proteins encoded by *rpo*A and *rpo*B accumulate in a 2:1 ratio (Igloi and Kössel, 1992), and the ribosomal proteins encoded by *rps*16 and *rpl*16 probably accumulate stoichiometrically. This observation led us to previously suggest that accumulation of the *rpo*A and *rps*16 proteins is

regulated at the level of translation or protein turnover (Baumgartner et al., 1993). Furthermore, the low levels and rapid changes in *rpo*B and *rps*16 mRNAs suggest that expression of these genes may modulate the rate of synthesis of the plastid-encoded RNA polymerase and plastid ribosomes.

Mature chloroplasts contain less stable proteins such as D1, the reaction center protein of PSII, which is damaged during photochemistry (Barber and Andersson, 1992). This protein is degraded and must be resynthesized to maintain photosynthetic capacity in chloroplasts. Mature chloroplasts, therefore, selectively maintain the capacity to translate D1. As previously reported, high *psb*A mRNA levels were present in chloroplasts of fully developed leaves of white-light-grown plants. The presence of elevated levels of *psb*A mRNA in mature pea leaves must by due to high RNA stability because *psb*A shows little differential transcription in white-light-grown plants. In barley chloroplasts of illuminated plants, the half-life of *psb*A mRNA was greater than 40 h (Kim et al., 1993).

In general, RNA levels corresponding to the genes encoding the plastid transcription and translation apparatus decreased in white-light-grown plants between 5 and 7 dpi, whereas mRNAs encoding proteins for the photosynthetic apparatus increased. Analysis of transcription during development of chloroplasts in white-light-illuminated plants showed that transcription of genes encoding the transcription/translation apparatus increased 2-fold relative to genes encoding the photosynthetic apparatus from 4 to 5 dpi and then decreased 8- to 10-fold from 5 to 9 dpi. This change is consistent with the need to synthesize the plastid's decoding apparatus early in chloroplast development and to utilize this capacity to synthesize proteins of the photosynthetic apparatus during the latter phase of chloroplast development. A similar broad-based change in transcription was previously documented in barley (Baumgartner et al., 1993). The change in transcription could be due to altered levels or activity of the nuclear-encoded plastid RNA polymerase relative to the plastid-encoded RNA polymerase. Alternatively, σ-like factors could modulate this change in plastid transcription activity (Tiller et al., 1991).

Differential transcription of plastid genes encoding the plastid's transcription and translation apparatus versus genes encoding proteins involved in photosynthesis during chloroplast development was previously documented in barley (Baumgartner et al., 1993). In this paper, we provide evidence for similar regulation in pea. This suggests that differential transcription of plastid genes during primary leaf and chloroplast development in higher plants may be common. Earlier studies in which differential transcription of plastid genes was not observed may have missed this phenomenon because a limited number of genes or stages in primary leaf development were analyzed.

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